AN ANTI-IDIOTYPE VACCINE FOR AIDS BASED ON THE HIV RECEPTOR

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Summary. - Idiotype studies have been important in our study of the HIV/CD4 interaction. They have predicted the binding site on CD4 and showed that it is the same amongst all HIV-I isolates, and therefore a formidable candidate for the design of effective therapeutic and vaccine strategies against HIV. Future research could implicate an anti-idioype approach to a prophylactic vaccine. It is important to note that an anti-idioype response may be needed to prime an immune response against a viral epitope which may be used as a secondary component. The use of anti-idioype responses in infected persons may be able to tip the balance of the immune system so that the body is able to control HIV infection. Until recently the use of anti-idioype approaches in the design of HIV vaccines has been sadly neglected.

KEY WORDS: anti-idioype, HIV receptor, vaccine.

Introduction

HIV causes disease in years rather than months in vivo. However, most virus infections cause an acute disease which is followed by an effective immune response in the majority of individuals. Some viruses are not completely cleared but may remain in a persistent infectious state and may be reactivated many years later, e.g. Herpes viruses. HIV may also cause an acute glandular fever-like illness which may be associated with viral antigenemia following which the patient becomes asymptomatic and HIV antigen is no longer detectable. The patient then remains well for several months to several years during which time the immune response which is initially normal, gradually deteriorates. It is hard to be sure which component of the immune response breaks down first but the central role of the HIV susceptible CD4 positive helper cells and macrophages in the orchestration of the immune response suggests that these cells are affected first [1]. This would affect antigen presentation and indeed this is one of the first abnormalities to be detected. Abnormalities of all aspects of the immune system have been described in HIV infected patients [2]. Importantly CD4 function appears to be impaired before the number of CD4 cells starts to fall. Moreover, the number of cells infected with HIV as shown by in situ hybridisation has been shown to be in the order of 1 infected cell to 10^3. This suggests that the immune deficiency seen before terminal AIDS is not due to the cytopathic effects of HIV.

Obviously in the terminal stages of HIV infection when the immune responses has collapsed, the uncontrolled replication of HIV probably plays a major cytopathic role.

Unlike HTLV-I and HTLV-II the strong serological response to HIV is not associated with a strong neutralising response or a strong antibody dependent cell mediated
cytotoxic response. The neutralisation response is very isolate specific although some broadening of the immune response is seen with some human sera [3]. A specific neutralising site has recently been described which is very isolate specific [4]. A single amino acid change will allow escape from neutralisation [5]. The envelope of HIV has been shown not only to be highly variable between isolates but also to vary with time [6].

The neutralising response is mainly to the envelope of HIV. Attempts to exploit this response to the envelope for the development of a vaccine, have unfortunately encountered the problems which are predictable from the facts given above. Developing a vaccine against HIV is fraught with problems. Should it be primarily humoral or cell mediated directed? (or preferably both). If the whole virus, live or inactivated, does not protect, what components should be selected? How should that be prevented? Can one protect an animal model from challenge? and if so will it be relevant to the human situation and finally how long will it take before a promising candidate can be shown to be useful in human populations. It is possible that envelope based vaccines could make the patient more susceptible to disease by antibody enhancement [7]. It is also possible that envelope based vaccines might induce a mild immunodeficiency per se.

A conserved receptor for HIV

Clinically it was noted that CD4 cell numbers fell following HIV infection. This is not unique to HIV as this scenario is seen in a variety of other infections. However, in vitro studies demonstrated cytopathic effects on CD4 positive cells which could be blocked by anti-CD4 antibodies [8, 9].

The evidence that the CD4 antigen is the receptor for HIV-I is compelling: a) many anti-CD4 antibodies block infection in vitro [8]; b) the CD4 gene transected into human cells renders them infectable to HIV [10]; c) HIV co-immunoprecipitates CD4 and no other cellular gene [11]; d) gp120 binds to CD4 with high affinity [10, 12]; e) following binding, fusion occurs at the surface and does not require endocytosis and phosphorylation [13].

Not only do all HIV-1 isolates use CD4 as their main if not sole receptor but so do HIV-2 and SIV [14]. Moreover, only part of the CD4 external domain is used for HIV binding [13]. This area has recently become the focus of much detailed research, the majority of which agrees that HIV-1 binding involves to the V, domain of CD4. Involvement of V, in contributing to binding or in post binding events cannot be ruled out at this time [15-19]. Moreover, the soluble component of CD4 is capable of binding and neutralising all known HIV-1 isolates to high titre [20], thus suggesting that this site may be the “Achilles heel” for HIV for therapy and vaccine purposes [21]. Indeed clinical trials with soluble CD4 have commenced at the NIH at the time of writing.

Early studies with available anti-CD4 monoclonal antibodies suggested that LEU 3a was one of the monoclonal antibodies that most clearly defined the gp120 binding site (which has been confirmed by subsequent studies) [22]. We therefore considered using LEU 3a as an immunogen to raise anti-idiotypic responses which should, in theory, represent the internal image of the binding site on CD4. This, in theory, should be able to bind and neutralise all HIV isolates.

Idiotype vaccines

The design of idiotype derived vaccines rests on the principle of molecular mimicking whereby an antigen can be substituted by an antibody possessing characteristics of that antigen. Anti-idiotypic antibodies (anti-Id) have been successful in inducing specific responses to a wide variety of foreign antigens including viruses in animal systems. A potential advantage of this approach of vaccination is that the use of a potentially dangerous pathogen or component thereof, is avoided.

Idiotype determinants are those determinants confined to the variable domains of an antibody molecule. A simple determinant is called an “idiotope”. Any exposed portion of the variable region of heavy or light chains may be potentially immunogenic. Initially anti-Id reagents were used to follow the inheritance of genetic markers on immunoglobulin variable regions and to map variable region genes. Jerne proposed that the vast repertoire of idiotopes interacted and played an important role in the regulation of the immune response so that an increase in an “Id” would induce an anti-Id response which could regulate production of the “Id”.

The antigen binding site of an antibody is known as the paratope, which in turn recognises the idiotope of some other antibody molecule. Therefore the epitope recognised by the first antibody AB, must largely overlap with the idiotope on the second antibody AB, and represent an internal image. These internal images could represent three dimensional structural configurations which represent the foreign epitope. It is this ability of the idiotype network that is so attractive to vaccinologists. Unfortunately and perhaps interestingly it is not that simple as non internal image antibodies (AB,) are induced which recognise non paratopic epitopes. The accepted terminology calls AB, which act as internal images of the antigen and which recognise the paratope of AB,-AB, and non-paratope idiotype induced AB, are known as AB, α if they recognise a framework associated idiotype or AB, if they recognise an antigen combining site related epitope which is not an internal image and is probably representative of intrastrain or interspecies id. Other AB, exist such as AB, E which will not be considered further here. Further details may be found in [23-26].

The AB, α response would be expected to be a nuisance in vaccine strategies aimed at raising the internal image of an epitope. However, the AB, α responses can activate clones which may be antigen specific so long as the AB, idiotopes for which they have specificity are present within the receptor repertoire (or V region sequences) of the responding animal.
AB response should therefore be strain specific whereas ABβ should not be restricted by genetic polymorphisms. It is interesting that many anti-idiotypic vaccine models have invoked protection by the ABα response.

Experimental models of idiotypic vaccines

A number of experimental models viral, parasitic, and bacterial infections have been developed to study the regulatory properties of the idiotype cascade.

Although the strategies for an HIV vaccine is being based on inducing an internal image (ABβ response), an ABα response has induced a neutralising response to the rabies virus. Studies with trypanosomiasis suggest that ABβ-like anti-Id may be genetically restricted in the induction of the immune response and may require the presence of a particular V region repertoire. The possibility of a Vβ-like anti-Id may have tremendous value based on the ability to activate normally silent clones.

Induction of an ABβ response has been successful in inducing a protective response against the surface antigen of HBV in chimpanzees [27]. This is an exceptionally exciting result as chimpanzees are the only animal to be susceptible to HBV apart from man. The ability to induce an internal image that functionally resembles an antigen has been shown for insulin, alpenolol, as well as non proteinaceous agents such as carbohydrates and phospholipid. This is particularly exciting for developing strategies which are dependent on tertiary structures such as the HIV binding site on CD4. Moreover, anti-Id has been produced against viral (Sendai) specific T cell clones (anti-cloneotypic) [28]. Relative to an HIV receptor based vaccine the anti-Id approach has identified the receptor for the reovirus [29].

Implications for an HIV vaccine

A monoclonal antibody which recognised the HIV binding site on CD4 could induce an ABβ response which represented the internal image of CD4. Soluble CD4 (which will contain this site) effectively neutralises a broad range of isolates [20]. The affinity of binding between gp120 and CD4 is in the region of 10^7 M. The same order of interaction has been found with soluble CD4 as well as with soluble subcomponents of the Vβ domain of CD4. In view of current problems with vaccines based on the envelope the ability to induce an internal image of the gp120 CD4 binding site may well be the best approach to the development of an AIDS vaccine [14, 21, 23].

Envelope based strategies were designed with the goal of inducing effective neutralisation in sera as well as a cell mediated response. Virus variability from isolate to isolate, the selection of neutralisation escape mutants in vivo, the presence of antibodies which enhance infection and the failure to protect chimpanzees from challenge has damped initial enthusiasm for this approach.

In theory the anti-idiotypic approach has the advantages that it is aimed at a highly conserved site vital for viral replication and that the immunogen does not contain any viral components. Unfortunately getting the ideal desired immune response is not easy. Of all the epitopes present on a mouse antibody only one is desired for the induction of an anti-HIV response. The body will see and make antibodies to all the other epitopes giving isotype, allotype and non-paratypic responses. Nevertheless, the schedule and method of presentation of an antibody as immunogen can influence the immune response to an antibody.

Early studies by P. Beverley and Q. Sattetant (I.C.R.F.) and T. Chanh and R. Kennedy (San Antonio, Texas) demonstrated a polyclonal as well as a monoclonal response to anti-LEU 3a in mice, which bound the immunogen as well as gp120 (albeit weakly) in addition to inhibiting syncitia and neutralising HIV [28]. A repeat study which used the immunisation schedule of Kennedy et al. based on that experience with Hepatitis B virus anti-idiotypic vaccines in chimpanzees was able to repeatedly induce an anti-anti-LEU 3a that inhibited fusion across a tangle of isolates including HIV-2. This study showed the immunisation schedule and the adjuvant to be critical in engendering an immune response [14]. Further studies in rats and rabbits and monkeys have repeated this response which unfortunately the ABβ response has been very weak as has the anti-HIV activity.

The above studies suggest that we may be using the wrong antibody which although near is not near enough the HIV binding site. A number of studies recently published has shown that the HIV binding site is in the Vβ domain of CD4 at amino acid numbers 38-52 [15-19]. Other sites may be involved in the overall interaction between gp120 and CD4 and the cell membrane. Of all the antibodies mapped to CD4, anti-LEU 3a best defines a site at position 42-43 where a mutational change destroys gp120 binding by greater than 90% (R. Sweet, personal communication).

This probably means that although very near, a better immunogen than LEU 3a is required. One such candidate is now under intensive study.

Recent studies on the CD4/gp120 interaction are optimistic in that the predicted binding site on CD4 does not lie in an immunoglobulin homologous region and is probably not involved in CD4 class II interaction [18, 19]. Together coupled with the neutralising data of soluble CD4 this data strongly underscores that this is the site on which future HIV vaccine development should be aimed.

Future studies will be aimed at making new monoclonals against CD4, defining the binding site with peptides and mimotopes. Three dimensional analysis of the binding site on CD4, making chimaeric monoclonal antibodies, trying combinations of immunogens, as well as an intensive study of adjuvants to be used in this approach which include alum, muramyl dipeptide derivatives and IL-2. Other more fancy delivery systems include coupling the right anti-CD4 component to anti-CD3 or anti-class II, as well as inserting the immunogen in a variety of liposomal vehicles.
Human studies

Encouraged by the initial animal studies we have begun a programme to examine the immune response to anti-LEU 3a given i.m. at weekly intervals without adjuvant to infected people (Walter Reed Stage 4). To date no ana-
phylactic or other early problems have been encountered.

Functional assays have not yet been completed. The first 3 patients have all made a good anti-idiotypic response which is encouraging as they would not be expected to respond well to new antigens with low CD4 counts and relative anergy. These trials are proceeding very cautiously as it is conceivable that a detrimental response could be induced as well as, or instead of the hoped for beneficial one.

The immune response in HIV infected patients

During our studies it became clear that the immune response in HIV infection is very restricted. This has been
recently confirmed by Grimaldi et al. [29]. This has important implications for vaccine strategies which study infected patients like the one above. It is possible that a strategy might be effective in infected patients but does not work as a protective vaccine or perhaps more likely a strategy which might induce protective responses in infected persons may be ineffective in infected patients.

If the response is clonally restrictive in infected persons then it may be possible to alter this restriction. One such strategy is to switch on silent or turned off responses and one way to do this is by idiotypic manipulation.

Future considerations

An important consideration with a potential vaccine preparation is cost per dose, together with safety and efficacy. Anti-Ids have been shown to be safe and effective in protecting a small number of chimpanzees against HBV. Chimpanzees are the relevant animal model for human HBsAg immunisation and HBV infection. Although the cost of producing a polyclonal anti-Id can be expensive, monoclonal anti-Id represents a means by which potential vaccine candidates can be produced in a cost-effective manner. A disadvantage of a monoclonal anti-Id when compared to its polyclonal counterpart is that the monoclonal anti-Id may possess the internal image of only a single epitope that is contained within a multideter-
minant antigen. If numerous epitopes are required to induce protective immunity against an infectious organ-
ism, a pool of different monoclonal anti-Ids may be necessary to induce complete immunity.

Alternatively, the anti-Id is a protein-based antibody molecule whose antigen-binding site (where the idiotype of Ab-1 binds) is composed of amino acids from the variable region heavy and light chains. It becomes possible to isolate homologous mRNA from a hybridoma produ-
cing the monoclonal anti-Id and, by using constant region heavy and light chain cDNA primers that are commercially available, sequence the V-region mRNA directly by the use of dideoxynucleotide sequencing. Another method for sequencing the variable region heavy and light chains employs reverse transcriptase to generate a cDNA analogous to the variable region mRNA and to isolate and sequence the cDNA. The amino acid sequence of the idiotype can then be deduced. By comparing other known immunoglobulin variable region sequences, along with computer programs that predict the most highly exposed areas within the sequences, it would be possible to make an educated guess as to which amino acid residues represent the mimicry site in the anti-Id primary structure. If a direct comparison between the primary structure of the antigen and the anti-Id is possible, regions of sequence homology could also be identified. The idiotope on the anti-Id that is responsible for the antigen mimicry may involve: amino acid sequences of the heavy chain alone; amino acid sequences of the light chain alone; or amino acid sequences from both heavy and light chains. With the first two possibilities, it would be relatively easy to utilise either recombinant DNA or synthetic peptide technology to generate an inexpensive synthetic anti-Id preparation. In the latter instance, one might anticipate that non-covalent interactions between the two individually produced heavy and light peptides might be strong enough to favour a conformation that, in solution, would mimic the antigenic determinant(s) on the pathogen. Recently, the nucleotide and deduced amino acid sequences of the heavy and light chains from an internal image monoclonal anti-Id have been reported. The anti-Id mimics the structure of the retrovirus haemagglutinin molecule. Comparison of the deduced amino acid sequence of the anti-Id with the retrovirus haemagglutinin revealed an area of significant homology present on the light chain of the anti-Id. This study suggested that antigen mimicry by antibodies may be achieved by the sharing of primary structure.

Anti-Ids have come a long way in a relatively short time in comparison with other technologies involved in an alternative approach for vaccination. However, further studies are necessary to determine the practicality and efficacy of this new generation of vaccines.

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